Development and Characterization of an Oro-Nasal Inhalation Plethysmography Mask Exposure System

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Abstract

An oro-nasal inhalation plethysmography mask exposure system (ONIPMES) was developed to challenge nonhuman primates and rabbits with biological agents while determining respiratory parameters in real-time. The system included novel challenge plethysmography and sample collection masks that delivered aerosol directly to the breathing zone of the animals and to the sample collection probes. Challenge plethysmography masks were fitted with a differential pressure transducer that interfaced with a signal amplifier and computer software to quantify respiratory tidal volume, frequency, and minute volume. Challenge plethysmography masks were calibrated and verified with certified registered gas-tight syringes. Accuracy was determined from simultaneous comparison tests between the challenge plethysmography mask and head-out plethysmographs using live animals. For cynomolgus macaques, the mean differences in tidal volume, frequency, and minute volume were 4.20 ± 0.872 mL, 3.50 ± 3.15 breaths per minute (bpm), and 99.3 ± 91.7 mL/min. For New Zealand white rabbits, the mean differences in tidal volume, frequency, and minute volume were 1.13 ± 0.551 mL, 1.07 ± 0.404 bpm, and 209.3 ± 97.37 mL/min. Standardized tests were used to characterize the inhalation exposure system. The fractional leak rate was 4.17×10^{-5} min^{-1}. The theoretical T_{99} was 1.7 minutes and the observed T_{99} was 0.6 minutes. Mask to mask spatial variation was 0.9%. The particle size distribution (PSD), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of a 25 mg/mL saline solution was 1.2 ± 0.01 µm and 1.9 ± 0.2. The ONIPMES minimizes dermal and ocular contamination and multiple species may be used.

Introduction

Oro-nasal (face) masks have been used for many years to deliver anesthetic gases (Popilskis and Kohn 1997) and test articles (Cheng et al., 2008) by inhalation to animals. Correspondingly, several methods are available to determine respiratory parameters in animals, including whole-body plethysmography (Lim et al., 2014), head-out plethysmography (body-only) (Obat Akata et al., 2007; Besch et al., 1996), and respiratory inductance plethysmography (RIP) (Khemani et al., 2015). There have been efforts to develop and refine the plethysmography equipment and procedures mentioned above to collect respiratory parameters in real-time during inhalation exposure. However, each of these systems present issues that may compromise plethysmography and study data integrity. For instance, whole-body plethysmography is generally practical for rodent species only and would introduce undesirable dermal and ocular contamination when used with nonhuman primates and rabbits. Furthermore, there is some discussion regarding the reliability of unrestrained whole-body plethysmography in some murine species (Alder, et al., 2004; Schwarze et al., 2005). Nonhuman primate head-out plethysmographs have been interfaced with head-only inhalation exposure chambers (Obat Akata et al., 2007), but these systems are cumbersome, space consuming, and may be prone to leakage. Moreover, commercially available rabbit head-out plethysmographs have slanted face plates and will not conveniently interface with head-only inhalation exposure systems. It should be noted that head-out plethysmography has routinely been conducted independently and just prior to inhalation exposure. However, as pointed out by Besch et al. (1996), results from this method may be highly variable and are dependent on the anesthetic used and the duration of anesthesia. Additionally, the animal would likely be in a different position

after being moved from the plethysmograph to the exposure apparatus, which could alter the measured tidal volume. Therefore, this procedure may not meet Good Laboratory Practice (GLP) requirements. Respiratory inductance plethysmography is a relatively new method of collecting real time respiratory parameters during nonhuman primate and rabbit inhalation exposures. This ventilatory system is calibrated using a face mask and evaluated using either an original linear model (OL), a nonlinear model of second degree (NL), or an alternative linear model (AL). The OL model is more accurate than the AL model and both are more appropriate than the NL model. However, the OL model has been found to underestimate lung volumes at the start of inspiration and overestimate lung volume at the end of inspiration in human patients due to the approximation of the 2 degrees of freedom of motion of the respiratory system (Strömberg et al., 1993). It is not clear if this artifact is present when used with animal models. Additionally, the accuracy of respiratory parameters, as derived from thoracic and abdominal bands, is subject to natural variability in the relative ribcage-to-abdomen contribution (Strömberg, 2001), placement on the subject (Mazeika and Swanson, 2007) and supine versus sitting positional effects (Strömberg et al., 1993). Another limitation of RIP technology, from an inhalation exposure point of view, is that the system must be calibrated for each animal. For NHPs, this could significantly increase the time the animal is under anesthesia. In the case of rabbits, this could significantly increase the time the animal is restrained.

The ONIPMES was designed to combine inhalation exposure with real-time plethysmography. This platform eliminates the need for head-out plethysmographs and RIP; it minimizes dermal and ocular contamination. Challenge plethysmography masks

fitted with a differential pressure transducer and signal amplifier were calibrated using certified traceable gas-tight syringes. Simulated respiratory volumes of air were introduced into the mask and the resulting waveforms were recorded and analyzed with and without an aerosol present. To confirm the functionality of collecting respiratory tidal volume and frequency data, side-by-side comparative tests between the challenge plethysmography mask and head-out plethysmographs were conducted.

The system was characterized using a battery of standard tests in order to demonstrate the ability to generate and deliver conditioned, respirable aerosol to the challenge plethysmography and sample collection masks. An aerosolized saline solution was used to determine time-to-99% steady state equilibrium concentration (T_{99}), spatial variation (% coefficient variation) between the challenge plethysmography and sample collection mask aerosol concentrations, and the aerosol particle size distribution (PSD) at the masks. This study describes the components, procedures and results from the development of this novel system.

Materials and Equipment

The ONIPMES was specifically designed to provide a single platform that would support multiple species, multiple test system concurrent inhalation exposures, and real-time plethysmography capabilities. The system consists of plethysmography and inhalation exposure components that share a common mask. Plethysmography instrumentation included an amplifier, differential pressure transducer and software (USB_AMP_4BR, DP_T, and IOX2 version 2.5.9.68; emka Technologies, Falls Church, VA), and custom designed challenge plethysmography masks. The inhalation exposure

component was comprised of an aerosol generation, conditioning and delivery line, a 12-port flat plenum (Model 03-104; In-Tox Products, LLC, Albuquerque, NM), an aerosol sampling platform, and an air handling station. The Collison Nebulizer was used for characterization tests (CN-24 Collison 3-jet Modified MRE Type and CN40 Glass Precious Fluids Bottle; BGI, Inc., Waltham, MA). The aerosol conditioning and delivery line consisted of a high efficiency particulate absorbing (HEPA) demand dilutor, an in-line radial mixer (Model 05-200; In-Tox Products, LLC., Albuquerque, NM) and a Quad Track Diffusion Dryer (Model 05-500; In-Tox Products, LLC., Albuquerque, NM). Conditioned aerosols were directed to a 12-port plenum and evenly distributed to one or two challenge plethysmography masks and two sample collection masks. All masks were size 5 canine anesthesia masks (DRE Veterinary, Louisville, KY) and had a volume of 0.593 L. The challenge plethysmography masks were fitted with an 8 mil dental dam gasket on the base and a pressure port mount that interfaced with the DP_T. A section of 15 mm pediatric anesthesia circuit (Model 921413; VetEquip, Inc., Pleasanton, CA) was connected to the apical end of the challenge plethysmography mask and to an aerosol delivery port on the plenum. The bases of the sample collection masks were sealed with a 0.25 inch Plexiglas® plate and fitted with a 0.25 inch diameter stainless steel sample collection probe that extended 2.0 inches inside the mask. The two exhaust ports were fitted with 3/8 inch tubing connectors and a 3/8 inch T-fitting. The common line from the T-fitting was connected to an exhaust port on the plenum. The apical end of the sample collection mask was also fitted with a section of 15 mm pediatric anesthesia tubing and connected to an aerosol delivery port on the plenum. Challenge plethysmography and sample collection masks are shown in Figure 1. The

aerosol sampling platform consisted of an Aerodynamic Particle Sizer (Model 3321 (APS) Spectrometer; TSI, Inc., Shoreview, MN), which was interfaced with one sample collection mask. The APS samples were pre-diluted using an Aerosol Diluter fitted with a 100:1 dilution capillary (Model 3302A; TSI, Inc., Shoreview, MN). Filtration samples were collected from the second sample collection mask to quantify aerosol concentration using in-line 25 mm filter holders (Model 06-100; In-Tox Products, LLC., Albuquerque, NM). When necessary, multiple filter samples were collected simultaneously from four sample collection masks using a custom designed manifold with 2 L/min (nominal) critical orifices. The air handling station used vacuum and compressed air gas flow controllers (Models MCPS-100 SLPM and MC-30 SLPM; Alicat Scientific, Tucson, AZ) operated in analog mode. A schematic of the ONIPMES is shown in Figure 2.

Procedures

Plethysmograph Calibration and Testing

To provide proof of concept that a pressure signal could be collected from a ventilated challenge plethysmography mask and integrated into a respiratory waveform, a certified calibrated 100 mL gas-tight syringe (Model CR1311; Hans Rudolph, Inc., Shawnee, KS) was used. The syringe was connected to the sealed end of the mask using a Luer-Lok fitting. The challenge plethysmography mask aerosol delivery line and exhaust lines were connected to the 12-port plenum. The plenum was ventilated at a volumetric flow rate of 12.0 L/min and a pressure of -0.4 in H_2O using a mass flow controller and configured to provide a flow rate of 4.0 L/min to the mask. For calibration,

a single 20 mL bolus of air was injected into the mask and the resulting impulse response from the pressure transducer was captured and processed by the IOX2 software. To simulate tidal breathing, 20, 40, and 60 mL (nominal) volumes of air were cyclically injected into and withdrawn from the mask. The same calibration was used for all three volumes of injected air. This range includes tidal volumes previously observed from cynomolgus macaques and New Zealand white rabbits. Respiratory waveforms were recorded and mean tidal volumes were determined.

To demonstrate that respiratory waveforms could be collected from the mask in the presence of an aerosol, the 12-port plenum was set up with a single challenge plethysmography mask and two sample collection masks. For this test, the plenum was ventilated at a flow rate of 15.0 L/min and a pressure of -0.6 in H_2O, which provided a flow rate of 5.0 L/min for each mask. The radial mixer was set at a flow rate of 5.0 L/min. A Model 3321 APS and Model 3302A Aerosol Diluter operating at 5.0 L/min were configured in series and connected to one sample collection mask. A 25 mm filter holder was connected to the second sample collection mask and operated at 2.0 L/min. A 25 mg/mL saline solution was made using S3014 sodium chloride (Sigma-Aldrich Corp., St. Louis, MO) and W9-1, Ultra Trace Elemental Analysis Water, (Fisher Scientific, Pittsburgh, PA) and placed in the Collison Nebulizer. The nebulizer was operated at 9.0 L/min. During system operation, calibration of the challenge plethysmography mask was conducted as described previously using a 20 mL bolus of air. Tidal breathing was simulated by injecting and withdrawing 40 mL (nominal) boluses of air. Respiratory waveforms were recorded and frequency, tidal volume and minute volume were determined.

Plethysmograph Comparison Testing

Animal research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

The challenge plethysmography mask was compared to nonhuman primate (NHP) and rabbit head-out plethysmographs in side-by-side concurrent tests using live animals. For cynomolgus macaques, a cylindrical plethysmograph (Model PLT_SC_PM; emka Technologies, Falls Church, VA) fitted with a DP_T was used. The cylindrical plethysmograph, with an internal volume of 34.9 L, was leak tested as previously described (Mokler and White, 1983). Nonhuman primates were sedated with 3 mg/kg Telazol and placed in the head-out plethysmograph in a supine position. The head of the animal was gently pushed through an annular orifice in an 8 mil dental dam gasket that formed a tight, gap-free seal around its neck without restricting breathing. After closing the cylindrical plethysmograph, the challenge plethysmography mask, fitted with a second DP_T, was placed over the snout of the nonhuman primate. Similarly, an 8 mil dental dam gasket with an annular orifice formed a tight, gap-free seal around the animal’s mouth and nose. For New Zealand white rabbits, a custom made trapezoidal head-out plethysmograph with an internal volume of 15.627 L and fitted with a DP_T was used. The rabbit plethysmograph was leak tested prior to use. Alert New Zealand

white rabbits were placed in Cat-Sack Restraints (Four Flags Over Aspen, Inc., St. Clair, MN) and positioned in a prone orientation in the plethysmograph. As with NHPs, an 8 mil dental dam gasket formed a seal around the neck of the animal. The challenge plethysmography mask, fitted with a second DP_T, was placed over the snout of the animal and formed a tight, gap-free seal using an 8 mil dental dam gasket. Both plethysmographs were monitored using IOX2 and calibrated with a 20 mL bolus of air as previously described. The dental dam gaskets of the plethysmographs and challenge plethysmography mask were visually monitored continuously during the tests to verify that they maintained a proper seal.

Aerosol Characterization

The ONIPMES was characterized to determine the integrity (fractional leak rate) of the system, T_{99} (time to ninety-nine percent steady state equilibrium concentration), spatial distribution and aerosol PSD. For aerosol characterization, the 12-port plenum volumetric flow rate was 15.0 L/min, the radial mixer volumetric flow rate was 5.0 L/min and the nebulizer volumetric flow rate was 9.0 L/min.

Plenum, Mask, and Anesthesia Circuit Integrity

In order to determine system integrity, the internal volume of the components was required. The internal volume of the 12-port plenum was determined by filling it with a known volume of water because its convoluted and complex design made direct measurement impractical. The combined volume of the three modified, size 5 canine anesthesia masks was determined by measuring a representative mask using the equation:

$$V = \frac{h\,\pi}{3}\left(r_{base}^2 + \left(r_{base} \times r_{top}\right) + r_{top}^2\right)$$

Where: h = mask height

r_{base} = radius of mask base

r_{top} = radius of mask top

The internal volume of the adult rebreathing circuit tubing was calculated using the equation:

$$V = l\,\pi\,\frac{d^2}{4}$$

Where: l = adult rebreathing circuit tubing length

d = inner diameter of adult rebreathing circuit tubing

System integrity was calculated by measuring the vacuum decay over time as described earlier (Mokler and White, 1983).

System T_{99}

The theoretical T_{99} was calculated using the total internal volume determined earlier, a system volumetric flow rate of 15 L/min, and the equation given by (Cheng and Moss, 1989):

$$T_{99} = -\ln\frac{100-99}{100} \times \frac{V}{Q}$$

Where: V = system internal volume

Q = system volumetric flow rate

The observed (actual) T_{99} was determined by collecting repeated APS samples of an aerosolized saline solution from a sample collection mask every five seconds. The Collison Nebulizer was filled with 10 mL of a 25 mg/mL saline solution. At t = 0 minutes,

the nebulizer and APS were initiated. After 10 minutes, the nebulizer was stopped and after 15 minutes APS sample collection was terminated. The APS log file generated by the Aerosol Instrument Manager (AIM v. 9.0.0.0) software (TSI, Inc., Shoreview, MN) was analyzed using SigmaPlot v. 12.5 (Systat Software, Inc., San Jose, CA). A time versus particle count plot of the saline aerosol data was made. Theoretically, the exponential rise and decay sections of the plot were mirror images. Therefore, the decay section of the curve was identified and isolated to facilitate the calculation of T_{99}. A SigmaPlot table was created in which the initial value was the point in the decay curve with the most particle counts and the time was set at zero minutes. Additional decay curve data were added to the table and the time was incremented by five seconds. Data points continued to be added until the particle count was less than 10. The resulting table was plotted and fit with an exponential decay, single, 2 parameter equation:

$$y = ae^{-kt}$$

Where: k = rate of change constant

t = time

a = constant

The observed T_{99} is given by the equation:

$$T_{99} = \ln 100 \times \frac{1}{k}$$ (Derivation in Appendix A)

Spatial Variation

The ONIPMES spatial variation was determined using the method described previously (Cheng and Moss, 1989). The system was configured with three sample collection masks and operated as described above for T_{99} determination. Each sample

mask was configured with a ¼-inch 316 stainless steel sampling probe that interfaced with a 25 mm filter holder. Pre-weighed Pallflex Type 61630 25 mm filters (Pall Corporation, New York, NY) were placed in each filter holder. Filter sampler volumetric flow rates were metered using custom designed 2 L/min (nominal) critical orifices. The critical orifices were installed in a custom designed sampling manifold that enabled individual or group sample collection. Each critical orifice was calibrated using a Primary Flow Calibration Device, Model 530 BIOS Defender (Mesa Labs, Inc., Lakewood, CO). Filters were weighed after collection and a mass per unit volume aerosol concentration was calculated. Temporal variation was determined from three independent reference samples collected from a single designated sample collection mask by calculating the aerosol concentration mean, standard deviation and percent coefficient of variation (%CV). Total variation was determined from samples collected simultaneously from each sample collection mask by calculating the aerosol concentration mean, standard deviation and %CV. Spatial variation was calculated using the equation:

$$CV^2_{spatial}\,(\%) = CV^2_{total}\,(\%) - CV^2_{temporal}\,(\%)$$

Particle Size Distribution

To determine PSD in the ONIPMES, saline aerosols generated from a 25 mg/mL solution were quantified using the APS and Aerosol Diluter fitted with a 100:1 dilution capillary tube. The aerosol particles were conditioned in transit using dilution air from the passive diluter and radial mixer and dried to a stable state using the Quad Track Diffusion Dryer. Aerosol samples directed to the APS were processed using AIM software and reported as count median aerodynamic diameter (CMAD), mass median

aerodynamic diameter (MMAD) and their respective geometric standard deviations (GSD).

Results and Discussion

Plethysmograph Calibration and Testing

The feasibility and accuracy of capturing and measuring respiratory waveforms from the ventilated, customized challenge plethysmography mask was determined. The prototype mask was calibrated using a 20 mL bolus of air injected using a 100 mL certified calibrated syringe. The mask was ventilated during the test to simulate actual use conditions. To simulate tidal breathing, 20, 40, and 60 mL volumes of air were cyclically injected into and withdrawn from the mask. The mean tidal volumes for each test are show in Table 1. Mean tidal volumes were 20.98 ± 1.300 mL, 41.85 ± 1.450 mL, and 60.66 ±1.280 mL. Simulated respiratory waveforms are shown in Figure 3.

The presence of aerosol in the system did not affect the accuracy of respiratory signals from the challenge plethysmography mask. Simulated tidal breathing using 40 mL boluses of air resulted in a 0.5 percent difference in the observed tidal volumes with or without aerosol present. The effect of aerosol on plethysmography data is shown in Table 2.

Plethysmograph Comparison Testing

Accuracy of the respiratory parameters was evaluated with direct comparison of mask and head-out plethysmographs in nonhuman primates and rabbits. For nonhuman primates, the mean difference in the observed tidal volumes, observed frequencies and

calculated minute volumes were 4.20 ± 0.872 mL, 3.50 ± 3.15 bpm, and 99.3 ± 91.7 mL/min (n=3), respectively. It was expected that the variances in tidal volumes, frequencies, and minute volumes would be less than those observed. However, all three nonhuman primates received 3 mg/kg Telazol which is less than the 6 mg/kg dose recommended by Besch, et al. and because of this they were waking up from anesthesia during the procedure. This may have resulted in a non-steady-state plane of anesthesia and adversely affected the data. Nevertheless, there was no significant difference in the observed tidal volumes between the challenge plethysmography mask and the cylindrical head-out plethysmograph ($p = 0.64 > \alpha$, $\alpha = 0.05$). Challenge plethysmography mask and head-out plethysmography direct comparison data are shown in Table 3. A representative nonhuman primate respiratory waveform from the challenge plethysmography mask is shown in Figure 4A.

For rabbits, the mean difference in the observed tidal volumes was 1.13 ± 0.551 mL (n = 3). The mean difference in the observed frequencies was 1.07 ± 0.404 breaths per minute. The mean difference in the calculated minute volumes was 209.3 ± 97.37 mL/min. There was no significant difference in the observed tidal volumes between the challenge plethysmography mask and the cylindrical head-out plethysmograph ($p = 0.93 > \alpha$, $\alpha = 0.05$). Challenge plethysmography mask and head-out plethysmography direct comparison data is shown in Table 4. A representative New Zealand white rabbit respiratory waveform from the challenge plethysmography mask is shown in Figure 4B.

Plenum, Mask, and Anesthesia Circuit Integrity

The internal volume of the 12-port plenum was 2.5 L, the internal volume of three masks was 1.779 L, and the internal volume of the anesthesia circuit was 1.239 L for a combined volume of 5.518 L. The initial test pressure was -2.24 in H_2O and the final test pressure was -2.15 in H_2O. The elapsed time for the test was 5.0 minutes. This resulted in a rate constant, k, of 8.2 x 10^{-3} min^{-1}, an estimated leak rate, Q, of 1.04 x 10^{-4} L/min and a fractional leak rate, $Q/V_c]_{1"}$, of 4.17 x 10^{-5} min^{-1}. $Q/V_c]_{1"}$ was well below the acceptance criteria of 0.001 min^{-1} established by Mokler and White (1983, p. 295) and the integrity test passed.

System T_{99}

Using the internal volume of 5.518 L and a volumetric flow rate of 15.0 L/min, the ONIPMES theoretical T_{99} was 1.694 minutes. Reduction of the data from the decay section of the time versus concentration plot produced a rate of change constant, k, of 7.8322 and an observed (measured) T_{99} of 0.588 min^{-1}. An observed T_{99} that is less than the theoretical T_{99} may indicate “short circuiting” of the aerosol through the system and the presence of dead space. Large amounts of dead space in larger systems may affect the time to reach steady state equilibrium and may increase the probability of different PSDs (Hemenway et al., 1982). However, it was anticipated that the small internal volume and stable PSD of the ONIPMES minimized these effects.

Spatial Variation

Spatial variation in the ONIPMES was measured using the temporal variation and total variation data obtained by filtration. For temporal variation, the mean filter sampler flow rate was 2.193 L/min and the mean sample volume was 65.79 L. The

mean aerosol concentration was 0.1203 ± 0.0006 mg/L (n=3) and the percent coefficient of variation (%CV) as 0.51. The mean filter sampler flow rate for total variation tests was 2.150 ± 0.010 L/min and the mean sample volume was 64.5 ± 0.28 L. The mean aerosol concentration was 0.0981 ± 0.0011 mg/L (n = 3) and the total variation %CV was 1.1. Spatial variation %CV was 0.9.

Particle Size Distribution

The PSD of NaCl aerosol generated from a 25 mg/mL saline nebulizer solution was determined by APS samples collection from a sample collection mask. PSD was presented as CMAD, MMAD and their respective GSD. The mean CMAD, GSD was 0.70 ± 0.01 µm, 1.3 ± 0.0 (n = 8). The mean MMAD, GSD was 1.2 ± 0.01 µm, 1.9 ± 0.2 (n = 8). Non-hygroscopic pharmaceutical and viable aerosols with a MMAD of 1.2 µm would be expected to have a pulmonary deposition fraction of 47% to 48% in nonhuman primates (Schlesinger, 1985) and 6% to 7% in New Zealand white rabbits (Raabe et al., 1988). Representative PSD plots are shown in Figures 5 and 6.

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Declaration of Interest

The views expressed here are those of the authors and do not necessarily represent the views or official position of the Department of Defense or the United States Army. Author L. Bowen is the inventor of the ONIPMES - patent application filed and pending. Author L. Bowen provides pro bono consultation services to In-Tox Products, LLC.

Appendix A.

$$\boldsymbol{C(t) = C_0 e^{-k \cdot t}}$$

$$\boldsymbol{C(t_1) = 0.01 \cdot C_0 = C_0 e^{-k \cdot t_1}}$$

$$\boldsymbol{\therefore 0.01 = e^{-k \cdot t_1} \Rightarrow ln(0.01) = ln\left(\frac{1}{100}\right) = -ln(100) = -k \cdot t_1}$$

$$\boldsymbol{\therefore t_1 = ln(100) \cdot \frac{1}{k} = t_{99}}$$

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Figure 1.

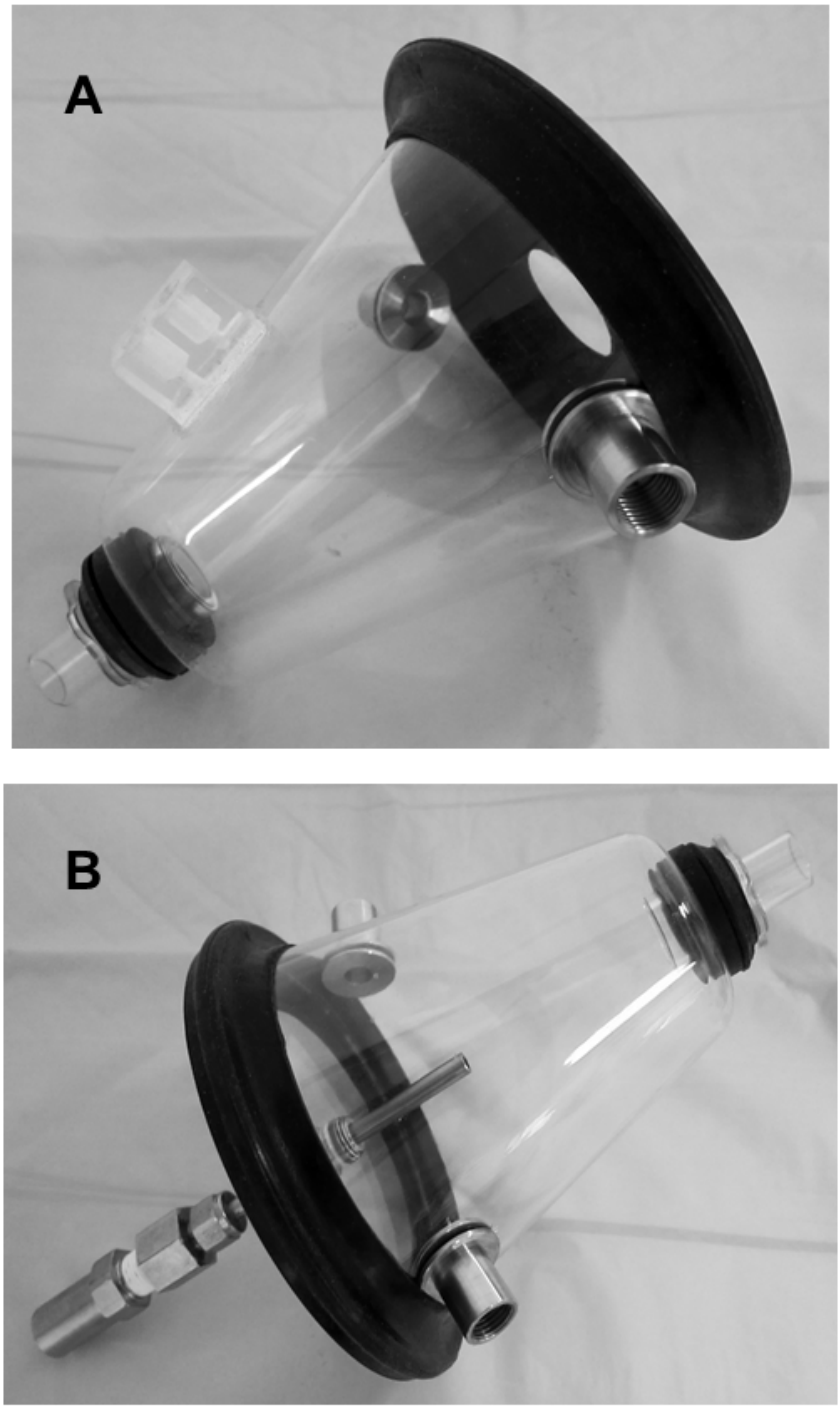

Figure 2.

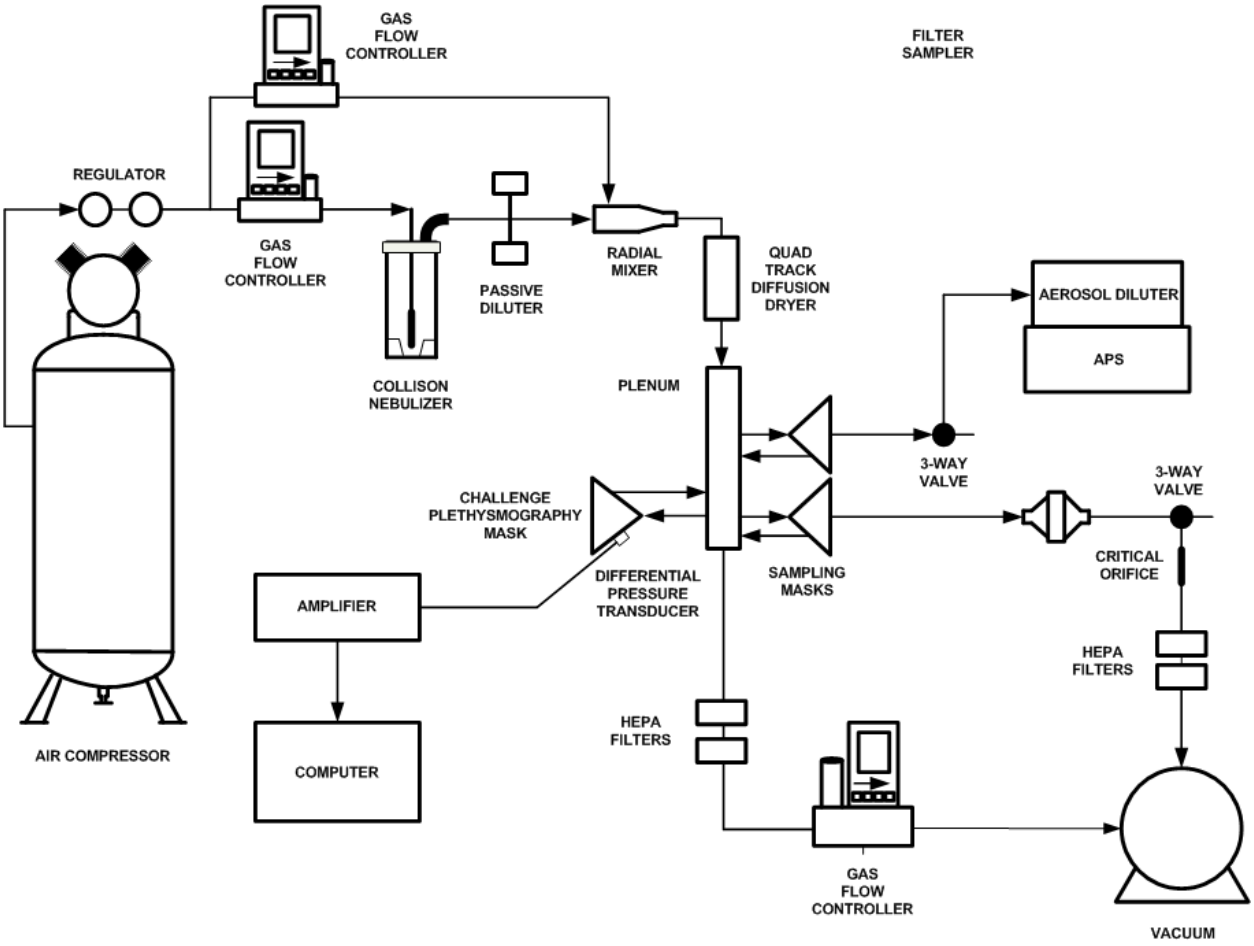
GAS FLOW CONTROLLER
FILTER SAMPLER
REGULATOR
GAS FLOW CONTROLLER
PASSIVE DILUTER
RADIAL MIXER
QUAD TRACK DIFFUSION DRYER
AEROSOL DILUTER
APS
COLLISON NEBULIZER
PLENUM
3-WAY VALVE
3-WAY VALVE
CHALLENGE PLETHYSMOGRAPHY MASK
CRITICAL ORIFICE
DIFFERENTIAL PRESSURE TRANSDUCER
SAMPLING MASKS
AMPLIFIER
HEPA FILTERS
HEPA FILTERS
AIR COMPRESSOR
COMPUTER
GAS FLOW CONTROLLER
VACUUM

Figure 3.

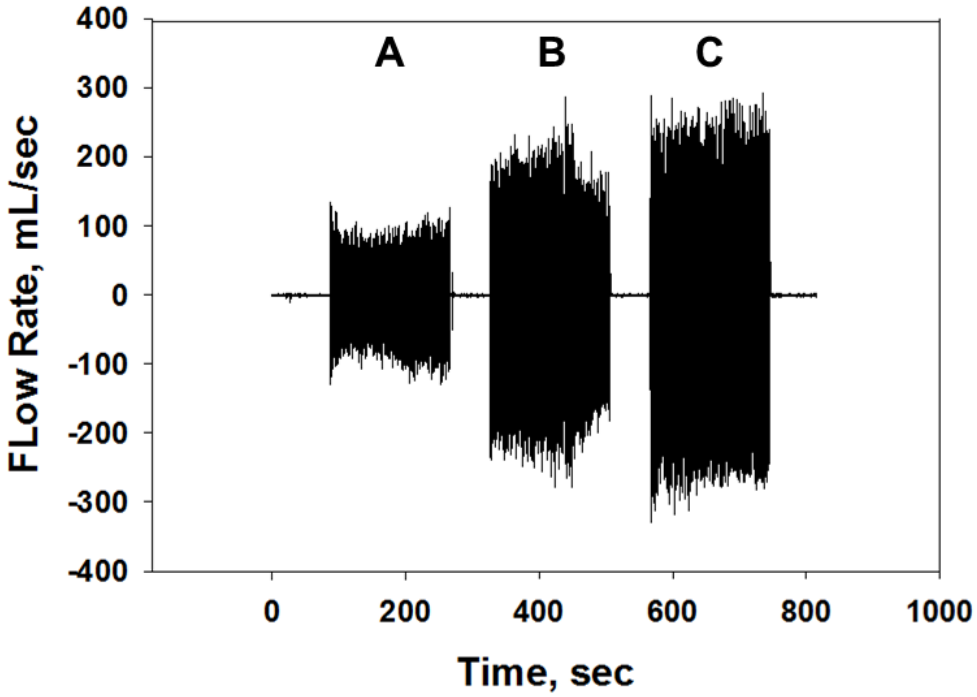
A
B
C
400
300
200
100
0
-100
-200
-300
-400
FLow Rate, mL/sec
0
200
400
600
800
1000
Time, sec

Figure 4.

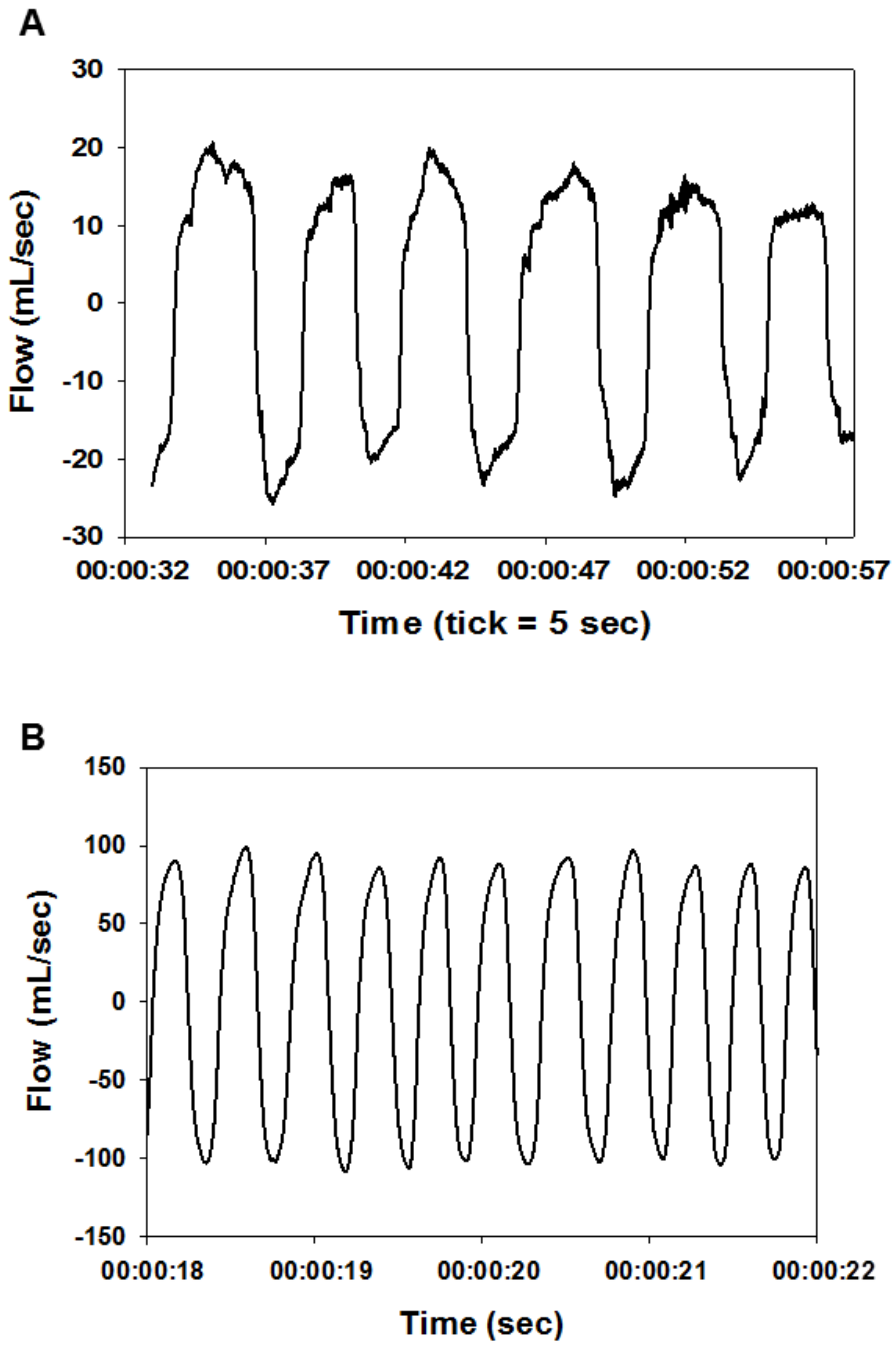
A
30
20
10
0
-10
-20
-30
Flow (mL/sec)
00:00:32
00:00:37
00:00:42
00:00:47
00:00:52
00:00:57
Time (tick = 5 sec)
B
150
100
50
0
-50
-100
-150
Flow (mL/sec)
00:00:18
00:00:19
00:00:20
00:00:21
00:00:22
Time (sec)

Figure 5.

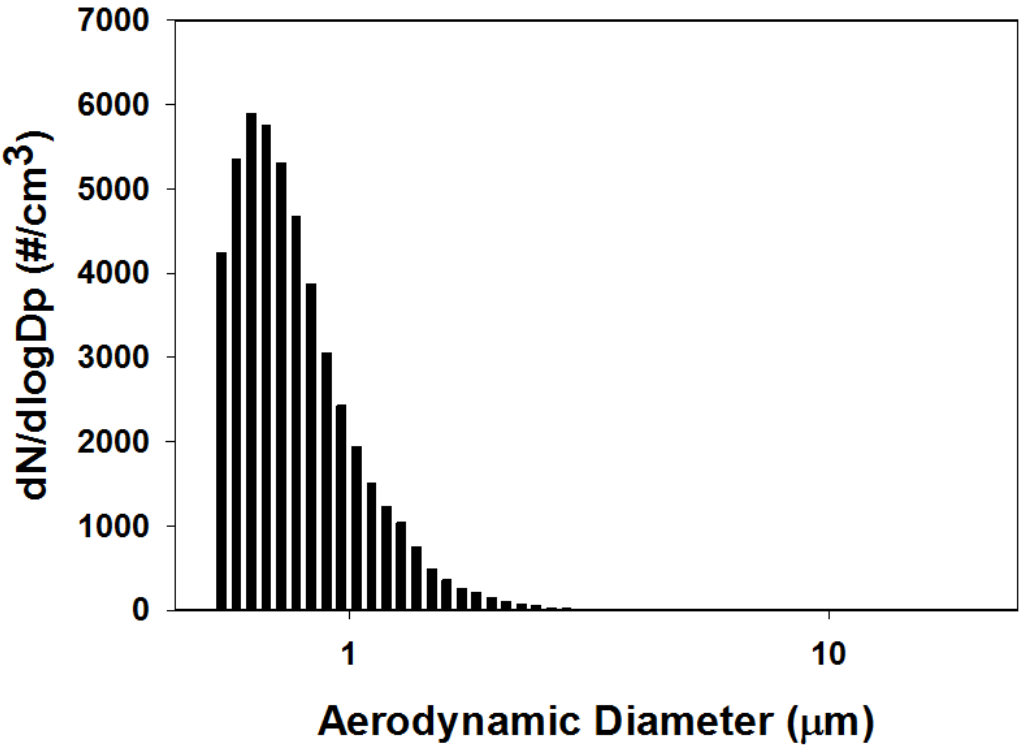
7000
6000
5000
4000
3000
2000
1000
0
dN/dlogDp (#/cm3)
1
10
Aerodynamic Diameter (μm)

Figure 6.

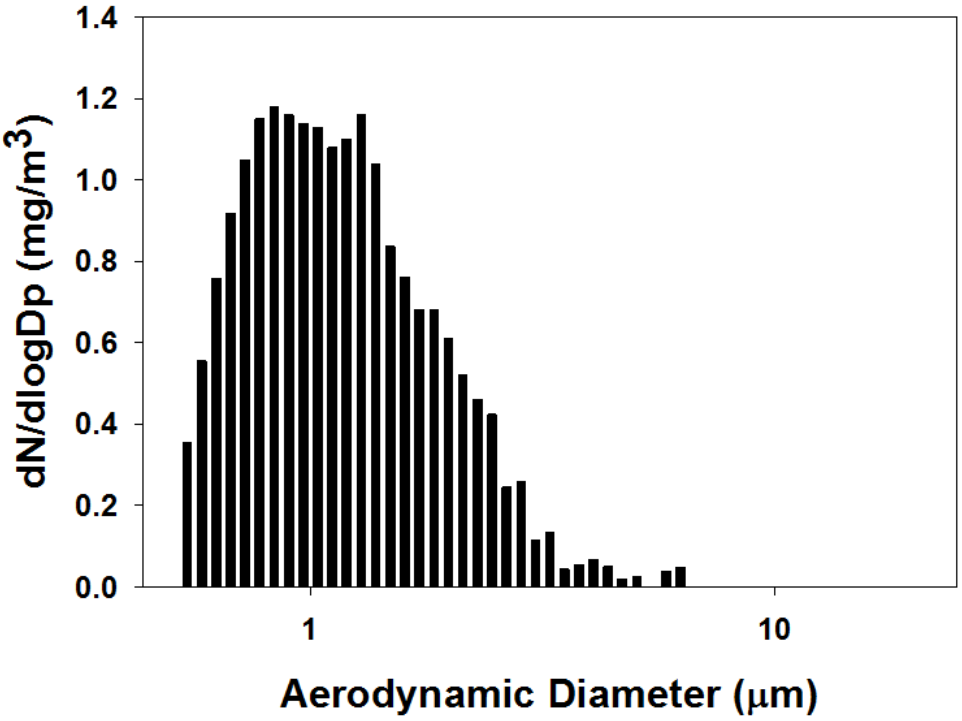
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
dN/dlogDp (mg/m3)
1
10
Aerodynamic Diameter (μm)

Table 1.

	Target Tidal Volume	Observed Mean Tidal Volume
	(mL)	(mL)
A	20	20.98 ± 1.300
B	40	41.85 ± 1.450
C	60	60.66 ± 1.280

Table 2.

	Mean Tidal Volume (mL)	Mean Frequency (bpm)	Mean Minute Volume (mL/min)
No Aerosol	40.62 ± 0.79	80 ± 9	3237 ± 317
Aerosol Present	40.42 ± 1.33	84 ± 8	3373 ± 255
Percent Difference	0.5	4.9	4.1

Table 3.

	Test ID	Tidal Volume (mL)	Frequency (bpm)	Minute Volume (mL/min)	Gender	Weight (kg)
Head-Out Plethysmograph	1	38.6	13.7	528.8	F	3.6
	2	20.1	31.1	625.1	M	8.2
	3	21.6	25.8	557.3	F	5.6
Challenge Plethysmography Mask	1	33.8	14.1	476.6	F	3.6
	2	15.5	27.1	420.1	M	8.2
	3	18.4	32.5	598.0	F	5.6
Difference	1	4.80	0.40	52.20		
	2	4.60	3.40	205.00		
	3	3.20	6.70	40.70		

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	26.76666667	22.56666667
Variance	105.5833333	96.74333333
Observations	3	3
Pooled Variance	101.1633333	
Hypothesized Mean Difference	0	
df	4	
t Stat	0.511426649	
P(T<=t) one-tail	0.317995039	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.635990078	
t Critical two-tail	2.776445105	

Table 4.

	Test ID	Tidal Volume (mL)	Frequency (bpm)	Minute Volume (mL/min)	Gender	Weight (kg)
Head-Out Plethysmograph	1	10.4	199.6	2075.8	F	4.8
	2	11.6	188.7	2188.9	F	5.2
	3	27.2	157.2	4275.8	F	4.3
Challenge Plethysmography Mask	1	11.0	199.0	2189.0	F	4.8
	2	9.90	190.0	1881.0	F	5.2
	3	26.1	155.9	4069.0	F	4.3
Difference	1	0.600	0.6000	113.20		
	2	1.70	1.300	307.90		
	3	1.10	1.300	206.80		

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	16.4	15.66666667
Variance	87.84	81.94333333
Observations	3	3
Pooled Variance	84.89166667	
Hypothesized Mean Difference	0	
df	4	
t Stat	0.097479777	
P(T<=t) one-tail	0.46351727	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.927034539	
t Critical two-tail	2.776445105	

Figure Legends

Figure 1. ONIPMES challenge plethysmography mask with differential pressure transducer mount (A) and sample collection mask with ¼-inch diameter probe (B).

Figure 2. The ONIPMES schematic demonstrating the configuration used during testing. Aerosol was generated using the Collison Nebulizer and conditioned using the passive diluter, radial mixer, and Quad Track Diffusion Dryer. Stable aerosol was directed to the 12-port plenum and was distributed to the challenge plethysmography mask(s) and sample collection masks. The animal's snout was placed inside the challenge plethysmography collection mask to receive aerosol. APS samples were collected from one sample collection mask and filter samples were collected from the other sample collection mask. Excess and exhaled aerosol was routed away from the plenum through a HEPA filter. The DP_T was connected to the challenge plethysmography mask and the amplifier. Airflows were metered using gas flow controllers.

Figure 3. Simulated respiratory waveforms were generated using a gas-tight syringe and collected from the challenge plethysmography mask. Volumes of (A) 20 mL, (B) 40 mL, and (C) 60 mL were repeatedly injected into the mask.

Figure 4. Challenge plethysmography respiratory waveforms from (A) nonhuman primate (B) New Zealand white rabbit.

Figure 5. Count PSD of a 25 mg/mL saline solution generated in the ONIPMES. The CMAD was 0.7 µm and the GSD was 1.3. Eighty-three percent of the number of saline particles were less than 1 µm and 99.9% were less than 3 µm.

Figure 6. Mass PSD of a 25 mg/mL saline solution generated in the ONIPMES. The MMAD was 1.2 µm and the GSD was 1.7. Forty-two percent of the mass of saline particles was less than 1 µm and 97% was less than 3 µm.

Table Legends

Table 1. Simulated respiratory waveform parameters (mean ± SD) from 20 mL, 40 mL, and 60 mL target injection volumes.

Table 2. Simulated respiratory parameters (mean ± SD) collected from a challenge plethysmography mask with no aerosol and with aerosol present. There was no significant difference in the observed mean tidal volumes from 40 mL injections ($p = 0.509 > \alpha$, $\alpha = 0.05$).

Table 3. Head-out plethysmograph and challenge plethysmography mask respiratory parameters direct comparison using nonhuman primates.

Table 4. Head-out plethysmograph and challenge plethysmography mask respiratory parameters direct comparison using New Zealand white rabbits.